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Synthesis and Anthelmintic Activity of 7-Hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides†

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Abstract: 7-Hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides represent a novel series of anthelmintic compounds, with broad-spectrum activity against important parasitic nematodes in sheep and dogs. In particular, an improved efficacy against *Trichostrongylus colubriformis* in sheep over the related 3-carbamoyl-4-hydroxycoumarins has been noted. New synthetic routes to the key intermediate, 7-hydroxythieno[3,2-*b*]pyran-5-one, have been developed.

Key words: anthelmintic, 7-hydroxy-5-oxothieno[3,2-*b*]pyran-6-carboxanilides/thiocarboxanilides, hydroxycoumarins

1 INTRODUCTION

The search for novel classes of therapeutic agent for the treatment of parasitic nematodes in domestic animals is of continuing interest due to the emergence of strains which show resistance to commercially available anthelmintics.[1] 3-Carbamoyl-4-hydroxycoumarins (**I**, Fig. 1) are known to possess insecticidal and anthelmintic properties.[2–5] These compounds usually show good activity against the intestinal nematode *Haemonchus contortus* Rud., but are generally less effective against *Trichostrongylus colubriformis* Giles. We have recently disclosed our findings concerning the novel thiophene

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† One of a collection of papers on various aspects of pest control research contributed by staff of Bayer AG and collated by Dr M. Londershausen.

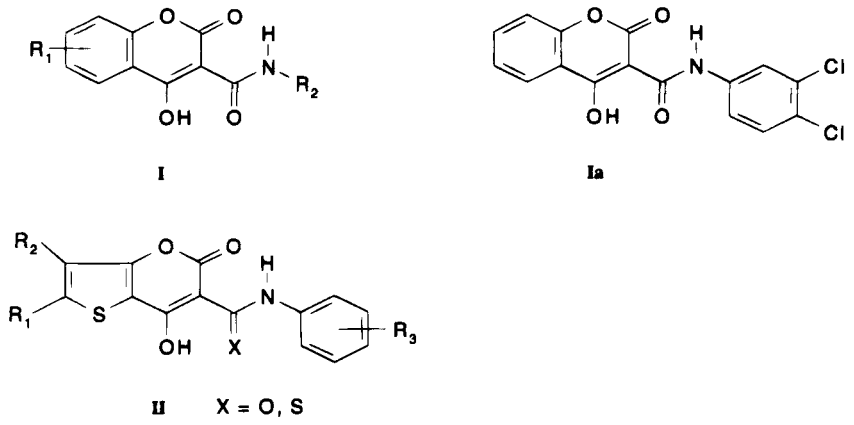

Fig. 1. 3-Carbamoyl-4-hydroxycoumarins **I**, **Ia** and structurally related thiophene analogues **II**.

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analogues mentioned in the title (**II**, Fig. 1),[6] and have observed improved biological activity against *H. contortus* and especially *T. colubriformis* in sheep. A preliminary account of this work was presented at the Eighth IUPAC Congress of Pesticide Chemistry, Washington DC, in 1994; in this paper we provide a more detailed account of the chemistry and biology of this novel class of anthelmintic agent.

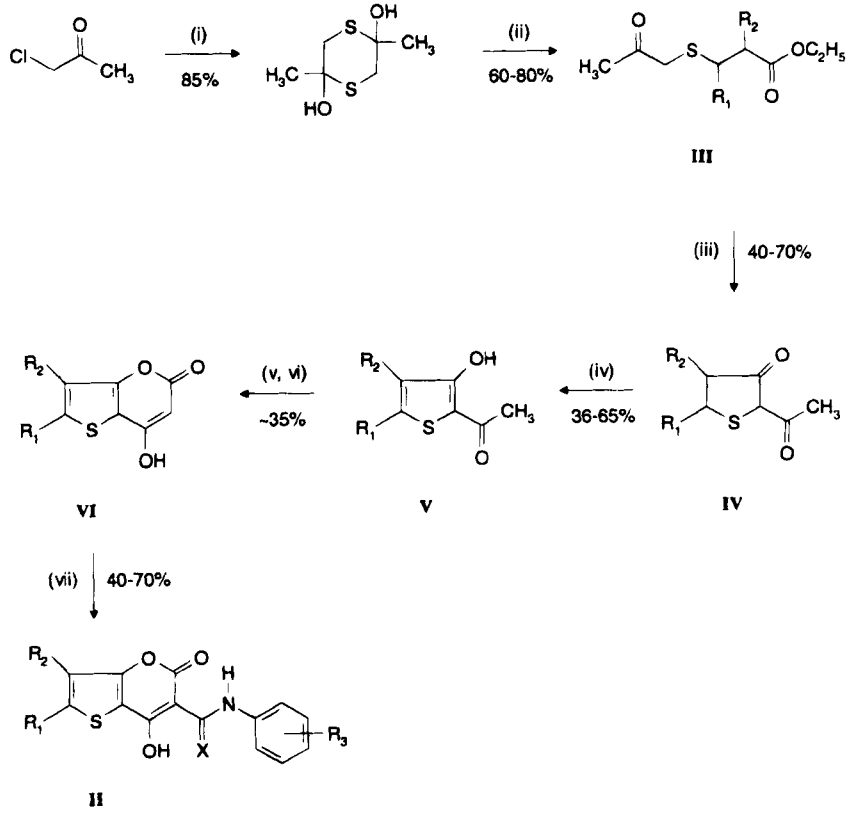
2 EXPERIMENTAL

2.1 Synthesis

Our first synthetic approach to 7-hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides (Fig. 1; **II**)[6] is outlined in Fig. 2. Acetonylmercaptan undergoes base-catalysed 1,4-addition to α,β-unsaturated esters, affording the dicarbonyl compounds **III**, base-induced ring closure of which gives the β-diketones, **IV**. These are subsequently dehydrogenated to the 2-acetyl-3-hydroxythiophenes **V** on reaction with sulfuryl chloride.[7] Heating such compounds under reflux in diethyl carbonate in the presence of sodium hydride, removal of all volatiles, and further heating under reduced pressure, produces the key intermediate, 7-hydroxy-thieno[3,2-*b*]pyran-5-one, **VIa**.[8] The title compounds **II** are formed on reaction of **VI** with iso(thio)cyanates under mild conditions.

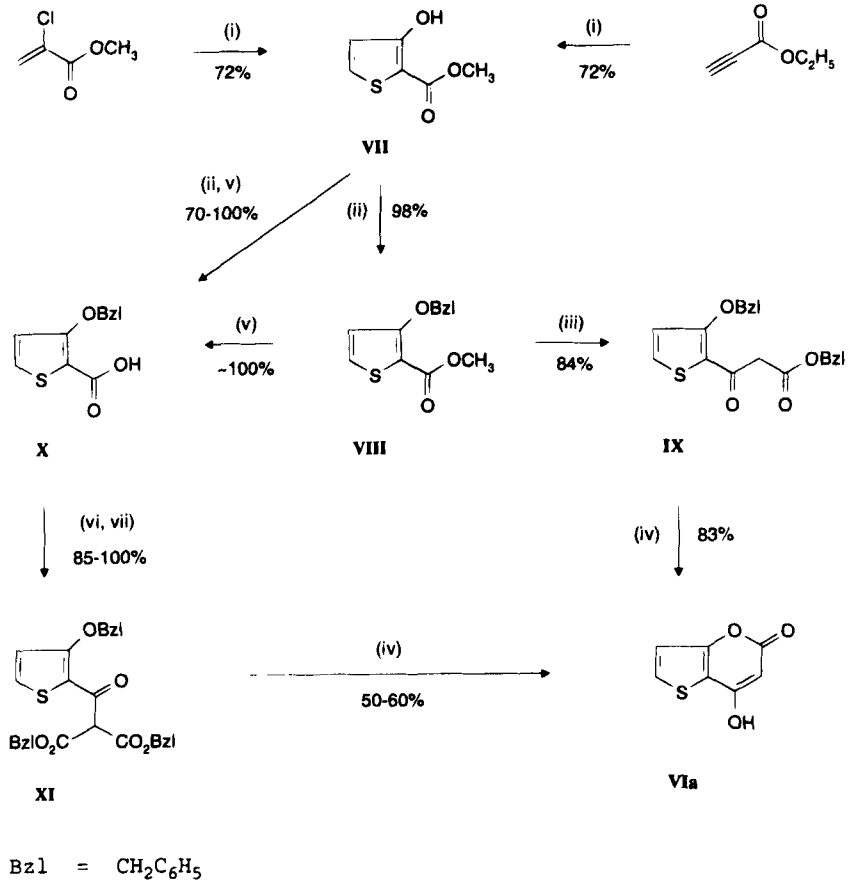
The disappointing overall yields (lowest when R^1 and R^2 = H) result primarily from the inherent tendency of compounds **V** to undergo acid-catalysed dimerisation and polymerisation reactions, due to their significant enol ether character. Consequently, improved synthetic routes to compounds **VI** were sought, particularly to 7-hydroxy-thieno[3,2-*b*]pyran-5-one (**VIa**, R^1 = R^2 = H), and these are depicted in Fig. 3.

Methyl 3-hydroxythiophene-2-carboxylate, **VII**, is prepared by reaction of methyl thioglycolate with either methyl 2-chloroacrylate[9] or ethyl propiolate under similar reaction conditions. Facile conversion to the



Reagents and conditions:
(i) Na_2S, HCl, H_2O, 0°C.
(ii) $R_1CH{=}CR_2CO_2C_2H_5$, piperidine (cat.), THF, 60°C.
(iii) $NaOCH_3$, $C_6H_5CH_3$, 25°C.
(iv) SO_2Cl_2, CH_2Cl_2, 25°C.
(v) NaH, $(C_2H_5O)_2CO$, reflux.
(vi) 160°C, ~2 mm Hg.
(vii) ArylNCX, 1,8-diazabicyclo[5,4,0]undec-7-ene, CH_2Cl_2 or DMSO, 25°C.

Fig. 2. Synthesis of 7-hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides, **II**.



Reagents and conditions:
(i) $HSCH_2CO_2CH_3$, $NaOCH_3$, CH_3OH, 25°C.
(ii) K_2CO_3, $C_6H_5CH_2Br$, acetone, reflux.
(iii) $CH_3CO_2CH_2C_6H_5$, $LiN[Si(CH_3)_3]_2$, THF, −78°C.
(iv) HBr, CH_3CO_2H, 60°C.
(v) NaOH, H_2O, reflux.
(vi) $SOCl_2$, reflux.
(vii) $CH_2(CO_2CH_2C_6H_5)_2$, $CaCl_2$, $(C_2H_5)_3N$, 60°C.

Fig. 3. New synthetic routes to 7-hydroxythieno[3,2-*b*]pyran-5-one **VIa**.

benzyl ether **VIII** is followed by reaction with benzyl α-lithioacetate to give the β-ketoester **IX** in very good yield and without the formation of carbinol by-products. Recrystallisation of the apparently pure (TLC, GC, NMR) ether **VIII** is essential to the success of this reaction. Debenzylation and cyclisation under acidic conditions produce the hydroxycoumarin analogue **VIa** (49% overall yield, four steps) *via* a route which compares favourably with our initial approach (6% overall yield, six steps). An alternative route to **VIa** more suited to the preparation of larger batches, and circumventing the use of organolithium reagents, begins with the hydrolysis of crude **VIII** to the carboxylic acid **X**. Conversion to the corresponding acid chloride and reaction with dibenzyl malonate under basic conditions gives the tricarbonyl compound **XI**. The target compound **VIa** is again obtained *via* acid-promoted debenzylation, cyclisation and decarboxylation (*c.*43% overall yield, six steps). Another noteworthy advantage of this approach is that purification of intermediates is not necessary in order to achieve a good overall yield.

2.1.1 General procedures

Reactions involving air-sensitive reagents were carried out under an inert atmosphere of argon. All solvents used were HPLC grade and were used without further purification. Melting points are uncorrected. All new compounds gave satisfactory spectroscopic and analytical data (C, H, N analyses). The instrumentation used, pertaining to the collection of analytical data, was as follows:- [^{1}H]NMR: XL 200 (Varian); FT-IR: FTS-60 (Biorad); MS (Finnigan): CI-MS: MAT 8340, FAB-MS: MAT 900, EI-MS: MAT 212. All yields reported are those of material which was judged to be homogeneous by TLC and NMR spectroscopy.

2.1.1.1 Methyl 3-hydroxythiophene-2-carboxylate, VII. To an ice cold solution of sodium methoxide (1·65 g,

30 mmol) in methanol (10 ml), was added methyl thioglycolate (2·12 g, 20 mmol) and the mixture was stirred at room temperature for 0·5 h. The solution was cooled to 0°C and ethyl propiolate (1·03 g, 10 mmol) was added dropwise; the mixture was then stirred at room temperature for 17 h, after which the reaction solution was acidified to pH 2 with hydrochloric acid (2 M) and extracted with dichloromethane (3 × 30 ml). The combined organic extracts were dried over magnesium sulfate, filtered and evaporated under reduced pressure, to afford **VII** as a mobile liquid which solidified on standing, (1·13 g, 72%) m.p. 43°C (lit.[9] 43°C); δ (deuterochloroform), 3·90 (3H, s, OCH_3), 6·74 (1H, d, J = 5 Hz, thiophene 4-H), 7·38 (1H, d, J = 5 Hz, thiophene 5-H), 9·55 (1H, broad, OH).

Alternatively, **VII** can be prepared with equal facility by the method of Huddleston and Barker.[9]

*2.1.1.2 Methyl 3-benzyloxythiophene-2-carboxylate, **VIII**.* A mixture of **VII** (47·40 g, 0·30 mol) and potassium carbonate (82·80 g, 0·60 mol) in acetone (150 ml) was stirred for 1 h at room temperature. Benzyl bromide (35·70 ml, 0·30 mol) was added and the mixture was then heated under reflux for 12 h. Acetone was removed under reduced pressure and the residue was taken up in dichloromethane + water (5 + 1 by volume; 600 ml). The organic layer was dried over magnesium sulfate and the solvent was removed under vacuum to afford **VIII**, as a viscous oil. Crystallisation could be induced by the addition of ice-cold diethyl ether (73·40 g 98%; 96·8% purity by GC); m.p. 66–67°C; IR (potassium bromide): υ_{ester} 1700 cm^{-1}; δ (deuterochloroform), 3·85 (3H, s, OCH_3), 5·25 (2H, s, CH_2), 6·81 (1H, d, J = 5 Hz, thiophene 4-H), 7·20–7·50 (6H, m, thiophene 5-H, Ar-H); FAB/MS *m/z*: 249 (M + H, 14), 91 (100).

*2.1.1.3 Benzyl 3-(3-benzyloxythiophen-2-yl)-3-oxopropionate **IX**.* *n*-Butyllithium (10·60 ml, 0·0266 mol, 2·5 M solution in hexane) was added dropwise to a solution of hexamethyldisilazane (5·50 ml, 0·0266 mol) in tetrahydrofuran (4 ml) at −78°C and the mixture stirred at this temperature for 0·25 h. A solution of benzyl acetate (1·90 ml, 0·0133 mol) in tetrahydrofuran (2 ml) was then added dropwise. Stirring was continued at −78°C for a further 0·5 h and then a solution of **VIII** in tetrahydrofuran (5 ml) was added slowly. The reaction mixture was allowed to attain room temperature slowly overnight and was then quenched with saturated ammonium chloride solution (10 ml). Hydrochloric acid (2 M) was then added until pH 3 was attained and the reaction mixture was extracted with dichloromethane (3 × 50 ml). The combined organic extracts were dried over magnesium sulfate, filtered, the solvent evaporated under vacuum and the residue recrystallised from dichloromethane-petroleum ether to afford **IX**, as a tan solid (3·98 g, 84%); m.p. 92–93°C; IR (potassium Bromide): ν_{ketone} 1620 cm^{-1}, ν_{ester} 1740 cm^{-1}; δ (deuterochloroform), 3·98 (2H, s, $COCH_2CO$), 5·09 (2H, s, OCH_2Ph), 5·11 (2H, OCH_2Ph), 6·82 (1H, d, J = 5 Hz, thiophene 4-H), 7·25–7·35 (10H, m, Ar-H), 7·55 (1H, d, J = 5 Hz, thiophene 5-H); FAB/MS *m/z*: 367 (M + H, 18), 91 (100).

*2.1.1.4 3-Benzyloxythiophene-2-carboxylic acid **X**.* Crude **VIII** (73·40 g, 0·295 mol) was dissolved in sodium hydroxide solution (1 M; 590 ml, 0·590 mol) and the solution heated under reflux for 2·5 h. It was then cooled, extracted with dichloromethane (2 × 100 ml) and the organic phases were discarded. The aqueous phase was acidified to pH 2 with hydrochloric acid (2 M), extracted with dichloromethane (2 × 200 ml), and the combined organic extracts were dried over magnesium sulfate and filtered. The solvent was then removed under reduced pressure. The residue was washed several times with ether to give **X** as a white solid (55·00 g, 80%); m.p. 125–126°C; IR (potassium bromide): υ_{co} 1666 cm^{-1}; δ (hexadeuterodimethyl sulfoxide) 5·26 (2H, s, CH_2), 7·13 (1H, d, J = 5 Hz, thiophene 4-H), 7·31–7·50 (5H, m, Ar-H), 7·71 (1H, d, J = 5 Hz, thiophene 5-H), 12·42 (1H, broad, OH); FAB/MS *m/z*: 235 (M + H, 60), 91 (100).

*2.1.1.5 Dibenzyl 2-(3-benzyloxythiophene-2-carbonyl) malonate **XI**.* The carboxylic acid **X**, (14·00 g, 0·06 mol) and thionyl chloride (9·20 ml, 0·13 mol) were heated under reflux for 1 h. After cooling, the excess thionyl chloride was removed under vacuum to afford the crude acid chloride. Dibenzyl malonate (17·07 g, 0·06 mol) and triethylamine (15·60 ml, 0·11 mol) were added dropwise sequentially to a suspension of calcium chloride (6·66 g, 0·06 mol) in acetone (74 ml) at room temperature. To the resulting suspension was added a solution of the acid chloride in acetone (5 ml) and the mixture was stirred for 12 h at 45°C. The reaction mixture was allowed to cool to room temperature, a solution of concentrated hydrochloric acid (5·2 ml) in water (16·0 ml) was then added carefully, and the mixture was extracted with dichloromethane (3 × 100 ml). The combined organic extracts were dried over magnesium sulfate, filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (eluent, cyclohexane + ethyl acetate 5 + 1 by volume), to give analytically pure **XI**, as a viscous oil (25·66 g, 85%); IR (KBr): ν_{ester} 1735 cm^{-1}, ν_{ketone} 1643 cm^{-1}; δ (deuterochloroform), 4·95 (2H, s, OCH_2Ph), 4·99 (2H, d, J = 12·5 Hz, CO_2CH_2Ph), 5·20 (2H, d, J = 12·5 Hz, CO_2CH_2Ph), 5·35 (1H, s, CH), 6·74 (1H, d, J = 5 Hz, thiophene 4-H), 7·19–7·38 (15H, m, Ar-H), 7·52 (1H, d, J = 5 Hz, thiophene 5-H); FAB/MS *m/z*: 501 (M + H, 1), 91 (100).

*2.1.1.6 7-Hydroxythieno[3,2-b]pyran-5-one **VIa**.* Compound **IX** (7·00 g, 0·02 mol) was added to hydrobromic acid in acetic acid (300 g $litre^{-1}$, 26·4 ml) and heated at 60°C for 5 h. The solution was allowed to cool and

diethyl ether (100 ml) was then added. The resulting precipitate was filtered and washed several times with diethyl ether, to give **VIa** as a tan solid (2·79 g, 83%); m.p. 225°C (decomp.) (lit.[8] 210–218°C); δ (hexadeuterodimethyl sulfoxide), 5·42 (1H, s, CH), 7·20 (1H, d, J = 5 Hz, thiophene 4-H), 7·99 (1H, d, J = 5 Hz, thiophene 5-H), 12·60 (1H, broad, OH).

Alternatively, **VIa** can be obtained by substitution of **XI** for **IX** in the above procedure.

2.1.1.7 4'-Difluoromethyl-7-hydroxy-5-oxo-5H-thieno [3,2-b]pyran-6-carboxanilide. (II, Table 1 entry 5). To a solution of **VIa** (15·12 g, 0·09 mol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (13·50 g, 0·09 mol) in dimethyl sulfoxide (120 ml) was added 4-difluoromethylphenyl isocyanate (15·21 g, 0·09 g) in one portion. After stirring at room temperature for 12 h, the reaction mixture was poured into water (450 ml) containing concentrated hydrochloric acid (27 ml) and the resulting precipitate was removed by filtration. It was then stirred in a mixture of methanol and dichloromethane (1 + 1 by volume 100 ml), re-filtered, and the remaining solid was washed with several portions of diethyl ether, to afford **5** as a beige solid (22·60 g, 74%); m.p. 200–201°C; IR: ν_{max} 3124, 1688, 1610, 1566, 1549, 1048, 1006, 795, 431 cm^{-1}; δ (hexadeuterodimethyl sulfoxide), 7·00 (1H, t, J_{HF} = 55 Hz, CHF_2), 7·37 (1H, d, J = 5 Hz, thiophene 4-H); 7·60 (2H, d, J = 8 Hz, Ar-H), 7·78 (2H, d, J = 8 Hz, Ar-H), 8·34 (1H, d, J = 5 Hz, thiophene 5-H), 11·25 (1H, s, OH); EI/MS *m/z*: 337 (M +, 32), 195 (22), 143 (100).

2.2 Biological assays

2.2.1 Evaluation of anthelmintic activity in vitro *using* Trichinella spiralis, *Owen, larvae and adult* Nippostrongylus brasiliensis, *Lane*

T. spiralis larvae were isolated from skeletal muscles and diaphragms of SPF/CFW1 mice and stored in sodium chloride solution (90 g $litre^{-1}$) supplemented with Canesten (20 $\mu g\ ml^{-1}$). The larvae (20 per estimation) were incubated in 2 ml of a solution with the following composition: Casitone 20; yeast extract 10; glucose 5; potassium dihydrogen phosphate 0·8 dipotassium hydrogen phosphate 0.8 g $litre^{-1}$ in water (500 ml; pH 7·2) and supplemented with Sisomycin (10 mg $litre^{-1}$) and Canesten (1 mg $litre^{-1}$).

The test compound (10 mg) was dissolved in dimethylsulfoxide (0·5 ml) and added to the incubation medium containing the larvae to give final concentrations of 1, 10 or 100 $\mu g\ ml^{-1}$. The mixture was incubated at 19°C for five days, after which activity was assessed according to Jenkins and Carrington[10] on a scale 1–3 where 1 = weak activity (fewer live larvae than in the untreated control); 2 = good activity ($\geqq$ 50% more dead larvae than in the untreated control) and 3 = full activity (all larvae dead).

N. brasiliensis adult worms were isolated from the small intestine of female Wistar rats and stored in sodium chloride solution (90 g $litre^{-1}$) supplemented with Canesten (2 mg $litre^{-1}$) and Sisomycin (20 mg $litre^{-1}$.) Worms (five male and five female) were incubated as described above according to the method of Rapson *et al.*[11] The resulting incubation medium was assessed for acetylcholinesterase activity, as performed by the same authors, using a scale 0–3 where 0 = no activity (< 50% enzyme inhibition) and 1, 2 and 3 weak, good and full activity (50–75, > 75 and 100% enzyme inhibition, respectively).

2.2.2 Mixed-parasite infections in mice

For all experiments male mice of the strain SPF/CFW1, 16–18 g body weight on receipt, were used. Five animals were housed per makrolon cage and given water and 'Sniff' rat feed, 13-cm pellets, *ad libitum*. Mice were mixed-infected with the tapeworm *Hymenolepsis nana*, Siebold, and the nematodes *Nematospiroides dubius* (= *Heligmosomoides polygyrus*), Dujardin, *Heterakis spumosa*, Schneider and *T. spiralis*. The infective material from *H. nana* was collected from mouse faeces 14–21 days post-infection (p.i.), third-stage larvae of *N. dubius* were collected from mouse faeces 21 days p.i., *H. spumosa* eggs were obtained from female worms isolated from the mouse colon 35–42 days p.i. and were then incubated at 27°C for three weeks, and *T. spiralis* larvae were obtained from pepsin-treated skeletal muscles and diaphragms of Wistar-W64 rats 20 days p.i. Test compounds (1 g) were dissolved or suspended in the emulsifier 'Cremophor' El (0·2 ml) and 0·5 ml of solution or suspension was administered per 20 g of mouse once daily for four consecutive days. One mouse received the highest dosage of 250 mg kg^{-1}; if anthelmintic activity was observed at this dosage, lower dosages of 100, 50, 25 and 10 mg kg^{-1}, respectively, were tested until anthelmintic activity could no longer be detected. The mixed-parasite infection was introduced into the mice in a stepwise manner. They were first infected orally with 90 embryonated *H. spumosa* eggs, followed seven days later with 100 *T. spiralis* larvae; 27 days later, 60–70 filariae from *N. dubius* were introduced and the final infection, with 100 *H. nana* eggs, followed after a further two days. Treatment began on day 46 p.i. and ended on day 49 p.i. After a further eight days the animals were killed with carbon dioxide and then dissected. *H. nana* were isolated from the small intestine and the number of tapeworms was estimated microscopically (magnification ×16). *H. spumosa* were isolated from the caecum and the colon, numbers also being determined by microscopy. Removal of the duodenum and treatment of this organ in a compressor, followed by microscopic examination (magnification ×40), allowed the numbers of remaining *N. dubius* to be determined. The numbers of *T. spiralis* present in *c.*1 cm^2 of abdominal muscle, removed by

dissection and compressed between two plastic sheets using a hand press, were determined by binocular microscopic examination (magnification ×40).

Activity against the three nematodes was evaluated on a scale 0–3 where 3 represents cure (no parasites detectable), 2 effective (<20% of parasites remaining), 1 trace effect (<50% of parasites remaining) and 0 ineffective (>50% of parasites remaining). Activity against the tapeworm was also evaluated on a scale 0–3 where 3 represents cure (no parasites detectable), 2 effective (some parasites expelled, tapeworm strobila excreted), 1 trace effect (tapeworm strobila excreted or macerated) and 0 ineffective (all parasites remaining).

2.2.3 Nematode infections in sheep

Sheep (Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *H. contortus* L3 larvae and treated with the test substance after the end of the pre-patency period of the parasite. The test compounds were administered orally in gelatine capsules. In the case of *T. colubriformis* and *Ostertagia circumcincta* Stadelmann, sheep were infected orally with 12000 and 20000 L3 larvae respectively, and similarly dosed with test compound. Anthelmintic effects of the test substances with respect to *H. contortus* and *T. colubriformis* were measured as a function of the reduction in the sheep faecal egg count. For the purpose of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel and the egg count was calculated per gram of faeces.[12] The egg counts were determined at regular intervals before and after treatment. The anthelmintic evaluation is expressed as a function of the egg reduction as follows: 3 = >95%, 2 = 75–95%, 1 = 50–75% and 0 = <50% egg reduction.

As the egg reduction test is not practicable for anthelmintic testing against *O. circumcincta*, the number of adult worms in the abomasum was directly counted on day 7 after treatment. The evaluation of the anthelmintic activity was carried out as for the egg reduction test.

2.2.4 Hookworm infections in dogs

Beagle dogs were each infected with 200–500 L3 larvae of *Ancylostoma caninum* Ercolani and *Uncinaria stenocephala* Raillet according to the published procedure.[13] After the end of the pre-patency period of the parasite, the dogs were treated orally with the test substance encapsulated in gelatine. To determine anthelmintic efficacy, the total daily faecal output of the dogs was examined for nematodes. After seven days of treatment, the dogs were treated with the anthelmintic 'Drontal Plus' (Bayer AG) and the daily faecal output re-examined for further expulsion of nematodes. The anthelmintic efficacy expressed as the percentage in nematode egg reduction was determined as described above in Section 2.2.3.

3 RESULTS AND DISCUSSION

3.1 Anthelmintic activity

The 7-hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides, **II**, tested *in vitro* were all found to be fully active against *T. spiralis* at 100 mg litre^{-1} (Table 1). The activity *in vitro* against the rat nematode *N. brasiliensis* was, however, variable. These initial results prompted studies using a mixed-parasite infection in mice. The 4-Cl derivative (Table 1, entry **1**) was amongst the best of a limited number of compounds tested in this model and had varying anthelmintic activity against the nematodes, examples being *N. dubius* (250 mg kg^{-1}, 3, full activity), *N. spumosa* (100 mg kg^{-1}, 3, full activity), *T. spiralis* (50 mg kg^{-1}, 0, inactive), and against the tapeworm *H. nana* (1 mg kg^{-1}, 3, full activity).

Encouraged by these initial results, compounds of structure **II** were then tested in sheep against the intestinal nematodes *H. contortus* and *T. colubriformis*, where they revealed their true potential (Table 1). Generally speaking, compounds **II** showed biological activity comparable with 3-carbamoyl-4-hydroxycoumarins **I** against *H. contortus*, whereas against *T. colubriformis* and *O. circumcinta* they performed significantly better. For example, compound **Ia**[4] (Fig. 1), a typical representative of the 3-carbamoyl-4-hydroxycoumarins **I**, was fully active against *H. contortus* at 5 mg kg^{-1}, but showed no anthelmintic activity whatsoever against *T. colubriformis* at 25 mg kg^{-1}. Noteworthy anthelmintic activity was also observed for compounds **II** against the hookworms *A. caninum* and *U. stenocephela* in dogs (Table 2). Thus, this new class of compound showed broad-spectrum anthelmintic activity against intestinal nematodes of both domestic and companion animals.

3.2 Structure–activity relationships

Derivatives of **II** substituted with a lipophilic electron-withdrawing substituent in the 4-position of the phenyl ring were generally fully active against *H. contortus* and *T. colubriformis* in sheep at doses of 2·5 mg kg^{-1} and 10 mg kg^{-1} respectively, (Table 1, entries **1–8**), with the 4-trifluoromethyl compound having additional anthelmintic activity against *O. circumcincta* (entry **4**). The 4-methyl derivative also showed good activity at 2·5 mg kg^{-1} against *H. contortus* and *T. colubriformis* (entry **9**). Introduction of the strongly electron-releasing 4-methoxy group resulted in a total loss of activity against *H. contortus* at a dose of 10 mg kg^{-1} (entry **10**). Moving the substituent to the 3-position in the phenyl

TABLE 1
Anthelmintic Activity of 7-Hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxylic acid phenyl(thio)amides **II**

						In-vitro Screening[a]		Anthelmintic activity in sheep	
Entry	R^1	R^2	R^3	*X*	*m.p.* (°C)	T. spiralis	N. brasiliensis	H. contortus	T. colubriformis
1	H	H	4-Cl	O	221	3[b]	3[c]	1[d]/3[e]	10/3
2	H	H	4-Br	O	228–229	3	2	2·5/3	10/2
3	H	H	4-F	O	220–221	3	1	2·5/3	10/0
4	H	H	4-CF_3	O	>250	3	2	1/3[f]	5/3
5	H	H	4-CHF_2	O	200–201	3	1	2·5/3	2·5/3
6	H	H	4-SCF_3	O	218–220	3	1	2·5/3	2·5/1
7	H	H	4-OCF_3	O	203–204	3	2	2·5/3	5/0
8	H	H	4-$OCHF_2$	O	178–179	3	1	1/3	10/3
9	H	H	4-CH_3	O	186	3	0	2·5/3	2·5/2
10	H	H	4-OCH_3	O	181–182	3	1	10/0	—[g]
11	H	H	3-CF_3	O	190	3	2	5/3	10/2
12	H	H	3-CH_3	O	169–170	3	0	10/1	10/2
13	H	H	2-CF_3	O	144–145	3	1	5/3	10/3
14	H	H	2-$OCHF_2$	O	184–185	3	0	5/0	—
15	H	H	2-CH_3	O	170–171	3	1	10/0	—
16	H	H	3,4-Cl_2	O	249–250	3	2	5/3	5/3
17	H	H	3-Cl, 4-SCF_3	O	188	3	0	2·5/0	2·5/3
18	H	H	3-Cl, 4-CF_3	O	240–241	3	1	1/2	5/3
19	H	H	3-Cl, 4-OCF_3	O	167	3	0	2·5/0	2·5/3
20	H	H	2,4-Cl_2	O	>250	3	0	5/3	10/1
21	H	H	2-Cl, 4-CF_3	O	230	3	3	5/3	5/3
22	H	H	2-CF_3, 4-Br	O	189–190	3	0	2·5/3	1/3
23	H	H	2-Cl, 4-OCF_3	O	187–188	3	1	2·5/3	2·5/0
24	H	H	2,3-Cl_2, 4-CF_3	O	242–243	3	2	10/3	—
25	H	H	2,6-Cl_2, 4-OCF_3	O	192	3	0	—	10/3
26	H	CH_3	4-SCF_3	O	221	3	1	10/0	10/0
27	CH_3	H	4-OCF_3	O	169	3	0	2·5/0	—
28	CH_3	H	4-$OCHF_2$	O	189–190	3	0	2·5/0	—
29	H	CH_3	3-Cl, 4-SCF_3	O	200–201	3	0	10/0	10/0
30	H	H	4-Cl	S	207–208	3	2	10/0	—
31	H	H	4-F	S	198–199	3	1	10/0	5/3

[a] Test compound concentration = 100 mg litre^{-1}.
[b] Anthelmintic activity was assessed according to Jenkins and Carrington[10] on a scale 1–3 where 1 represents a situation where there are less live larvae present than in the untreated control (weak activity); 2 where there are >50% more dead larvae present than in the control (good activity) and 3 where all larvae are dead (full activity).
[c] Anthelmintic activity was correlated with the degree of acetylcholinesterase activity,[11] using a scale 0–3 where 0 represents no activity (<50% enzyme inhibition) and 1, 2, and 3 weak, good and full activity) (50–75, >75 and 100% enzyme inhibition, respectively).
[d] Dose in mg test substance kg^{-1} body weight.
[e] The anthelmintic evaluation is expressed as a function of the faecal nematode egg reduction count[12] as follows: 3 = >95%, 2 = 75–95%, 1 = 50–75% and 0 = <50% egg reduction.
[f] Activity *in vivo* against *Ostertagia circumcincta* (10/2) has also been observed.
[g] Not determined.

ring gave compounds that were significantly less active against *H. contortus* (entries **11** and **12**), with a further decrease in activity being noted on shifting the group to the 2-position (entries **14** and **15**). The introduction of an additional 3-chloro substituent into the 4-substituted series had a mixed effect on the anthelmintic activity, with a reduction in efficacy against *H. contortus* and an increase in the biological activity against *T. colubriformis* being noted (entries **17–19**). The 2,4-di-substituted derivatives showed good activity against both *H. contortus* and *T. colubriformis*, with the 2-trifluoromethyl-4-bromo compound being amongst the most active substances tested (entry **22**). Trisubstituted derivatives were generally inactive against either of the aforementioned nematodes at doses of less than 10 mg kg^{-1} (entries **24** and **25**). Alkyl substitution in the thiophene ring resulted in a dramatic loss of activity (entries **26–29**) and thioamides were inactive against *H.*

TABLE 2
Anthelmintic Activity of 7-hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxylic acid phenylamides in dogs

Entry	R^1	R^2	R^3	X	*m.p.* (°C)	*Activity against* A. caninum	*Activity against* U. stenocephala
1	H	H	4-Cl	O	221	25[d]/3[e]	25/3
2	H	H	4-Br	O	228–229	25/3	25/3
3	H	H	4-F	O	220–221	25/3	25/3
4	H	H	4-CF_3	O	>250	—[g]	25/3
6	H	H	4-SCF_3	O	218–220	25/3	25/3
9	H	H	4-CH_3	O	186	25/0	—
20	H	H	2,4-Cl_2	O	>250	25/0	—

[d] Dose in mg test substance kg^{-1} body weight.
[e] The anthelmintic evaluation is expressed as a function of the faecal nematode egg reduction count[12] as follows: 3 = >95%, 2 = 75–95%, 1 = 50–75% and 0 = <50% egg reduction.
[g] Not determined.

contortus at 10 mg kg^{-1} (entries **30** and **31**).

Of the limited number of compounds tested in dogs (Table 2), no significant differences in the efficacies of those compounds bearing a range of lipophilic electron-withdrawing substituents could be detected. Full biological activity against the hookworms *A. caninum* and *U. stenocephala* was noted at a dose of 25 mg kg^{-1}. In summary, derivatives of **II** unsubstituted in the thiophene ring, containing an amide linker and bearing 4- or 2,4-substituents in the phenyl ring, represent the most anthelmintically active compounds in both sheep and dogs.

4 CONCLUSIONS

7-Hydroxy-5-oxo-5*H*-thieno[3,2-*b*]pyran-6-carboxanilides and -6-thiocarboxanilides, **II**, represent a novel class of anthelmintic agents with improved activity in domestic and companion animals as compared to 3-carbamoyl-4-hydroxycoumarins **I**. Mode of action and tolerance studies have yet to be completed.

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